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DETERMINATION OF METAL CONTENT IN TEA LEAVES GROWN IN YEN BAI AND TUYEN QUANG PROVINCE, VIET NAM

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ABSTRACT

The metal contents of 19 tea leave samples, *Camellia sinensis* L., from Yen Bai and Tuyen Quang provinces were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS). Total 18 elements in tea leaves were determined including macroelement such as Al, Ca, K, Mg, Mn; trace element such as Na, Ba, Fe, Cu, Sn, Zn, Se, Ni, B and heavy metal (Pb, Hg, Cd and metalloid As). The results show that, K content was the highest among the macroelement, followed by Mg, Ca, Mn and Al. There was a wide variation of the trace metal content among the tea leaves coming from different province. The levels of heavy metal content in most of the samples were not detectable or below the acceptable level as required by Ministry of Health.

Keywords: tea leaves, *Camellia sinensis* L, metal, trace element, heavy metal.

1. INTRODUCTION

Tea is the most popular beverage product in the world after water which is made from the leaves of a shrub *Camellia sinensis* L. Tea contains flavonoids, minerals, and trace elements that are essential to human health. There has been a lot of studies on the medicinal value and beneficial health effects of tea consumption. Some of the possible effects of tea is tea contains a lot of vitamins and antioxidants that prevent cancer and heart disease [1]. Tea can also prevent anorexia, good for digestion, help heat the body. In the context of global economic integration, tea processing and exporting enterprises are under pressure of technical barriers, especially the food safety issues of exported tea products. The safety of the finished product depends much from the primary production of the whole value chain. The determination of metal content in raw materials and finished tea samples also contributes to the safety and quality of finished tea products.

The metal content, including essential elements such as K, Ca, Mg, Mn, Al, the trace elements such as Fe, Cu, Mg, Zn of tea is not significant, however, the role of metal content is very important. The element content in tea leaves may depend on several factors such as variety of the plant, geographical location where the plant is cultivated, fertilizer, and industrialization process and storage conditions. It is well investigated on the content of different trace elements, heavy element and essential elements in different kinds of tea product from China, India, Brazil, Taiwan, Iran [2 - 6]. The knowledge of both macroelement, microelement or trace element and toxic element content in beverages as well as the raw material is important, taking into account nutrition requirement and intoxication risk related with their consumption [7]. The understanding of the content of those elements is of importance for the evaluation of the quality and safety of finished tea products. This paper intended to provide information about the content of essential and trace element and heavy metals in the tea leaves grown in Tuyen Quang and Yen Bai (young leaves and bud of *Camellia sinensis* L.) by using inductively coupled plasma optical emission spectrometry (ICP-OES) and atomic absorption spectrometry (AAS).

2. MATERIAL AND METHOD

2.1. Sample collection

Nineteen samples were randomly collected during December 2016 at different tea plantations in Yen Bai and Tuyen Quang provinces. The variety of these tea leaves were LDP1, which is the major variety that used as raw materials for green tea processing in the Northern region of Vietnam. Samples were taken in the form of fresh leaves (including young buds and young leaves which are suitable for processing of green tea product, herein can be called briefly as tea leaves). For fresh tea samples, to ensure that the sample is not damaged until analyzed, the sample is pre-dried at 50 - 60 °C before brought to analyze at the laboratory.

2.2. Sample preparation

Acid digestion of the tea samples was carried out before analysis. The tea leaves were grinded in small pieces before being brought to the homogenization of samples in the microwave digestion system CEM 5 (CEM Corporation, North Carolina, USA). An amount of about 0.2 g - 0.5 g was weighed to put into the sample container of the microwave oven. The sample was then added with 2.5 mL of 70% concentrated acid nitric HNO_3 and 2.5 mL of distilled water. The samples were digested in the furnace with the temperature 200 degrees C and pressure 400 psi during 45 min. After the inorganic process was completed, the sample was transferred to a 25 ml volumetric flask.

2.3. Determination of dry weight content

In order to compare the metal content of different samples, the calculation is based on the dry weight content of each sample. The dry weight content was determined by drying the samples to constant weight in dryer 1350FX -2CE at 105 °C until there is no change in the sample’s weight (TCVN 5613:2007).

2.4. Instrumental analysis

Analysis by ICP-OES: The metal content of the inorganic portion from all tea leave samples after treatment were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). An Optima 7300 DV ICP OES spectrometer (PerkinElmer, MA, USA) was used for elemental analysis. The operating condition was as the following: argon flow rate 16 L/min; Argon pressure 275 kPsi; repeated measurement: 15, and number of repeated measurement: 2. The analytical procedure was following the TCVN 6665:2011 [8]. Water purified in a Milli-Q system (Millipore, USA), was used throughout the work. Nitric acid and HNO3 of supargrade were from Merck (Damstadt, Germany). Calibration solutions were prepared fresh daily by serial dilution of working solution (100 mg/L) in HNO_3 solution. Stock solutions of multi-element for ICP were obtained from Merck (Germany) of 1000 mg/L

Determination of Hg, Pb and Cd by Atomic Absorption Spectrophotometric AAS: The determination of Hg was carried out using Varian 240 with mercury electodeless discharge lamp. The AAS equipment capable of continuum background correction was used in the analysis. $NaBH_4$ was used to reduce Hg (II) to metal Hg in HCl by Argon gas flow. The content of Hg was measured by AAS. The standard solution series were prepared from working standard solution in HCl solution 5 M and $NaBH_4$ (0.5 % of NaOH and 0.6 % $NaBH_4$). The sample was digested in CEM 5 and transfered to 25 mL volumetric flask. The measurement was done on AAS under the absorbance lengh at 253.7 nm.

Pb and As was determined by Graphite Furnace Atomic Absorption Spectroscopy(GFAAS) (Varian 240) after the digestion of the samples. The standard curve was developed based on standard solution series which is diluted from working standard solution of 100 ppb Pb and Cd. The stock solution is 1000 ppb. The absorbance length for measurement of Pb was 283.3 nm and As was 228.8 nm. In order to control the quality of the analyses, it was necessary to measure the known concentration of standard solution.

The validation of the analysis: Validation parameters were based on the limit of detection (LOD) and quantification (LOQ), linear range. LOD were calculated as 3 s/m, where s is the standard deviation of 10 successive injections of the blank and m is the slope of the calibration curve. LOQ were calculated as 10 s/m. Range of linearity was evaluated by checking the linear regression coefficient (R^2) of the calibration curve. The linearity of the calibration curve was considered acceptable when $R^2 > 0.995$.

3. RESULTS AND DISCUSSION

3.1. The accuracy of the analytical method

Quality control or the accuracy of the analytical method was done with the validation of the measurement based on the standard substances. Limit of detection (LOD), limit of quantification (LOQ) and coefficient of determination (R^2) are expressed in *Table 1*. The metal contents of the tea leave samples were determined by using the standard line constructed for each element.

3.2. Metal content in green tea leaves from Tuyen Quang and Yen Bai provinces

The range of element contents in tea leaves from Yen Bai and Tuyen Quang provinces are displayed on Table 2,Table 3, Table 4 for macroelement such as K, Mg, Ca, Mn and Al, trace elements (Na, Ba, Fe, Cu, Zn, B, Sn, Se, Ni) and heavy metals (Pb, Cd, Hg and metalloid As), respectively.

From these results, it can be seen that K, Mg, Ca, Al, Mn were the elements with the content above 200 mg/kg (Table 2). In this study, the most abundant element among the macroelements was K, followed by Ca, Mg and Mn. These elements contribute to the characteristic flavor of tea such as sweetness and aroma of tea. This result is similar to other report on tea leave in general [2]. K plays an important role in activation of enzymes relating to different biosynthetic processes of the tree, increasing the productivity and the sweetness of the finished made tea. Ca and Mg are the elements that contribute to build the cells of the leaves. Al and Mn also belong to the macroelements in tea leaves with the content varied from 320.07 mg/kg to 2149.15 mg/kg, and 237.96 mg/kg to 2454.48 mg/kg, respectively. This result is also in accordance with the report [7] indicating the accumulation of Mn in tea leaves was rather high.

Table 1. Validation of analytical method [8].

Metal	Wave length (nm)	Limit of Detection LOD (ppb)	Limit of Quantification LOQ (ppb)	Coefficient of determination (R^2)
K	766.490	0.5	1.5	0.999
Ca	317.933	0.5	1.5	0.997
Mg	285.213	0.5	1.5	0.999
Mn	297.322	0.5	1.5	0.999
Al	396.153	0.5	1.5	0.999
Ba	233.527	0.5	1.5	0.999
Fe	238.204	0.5	1.5	0.999
Cu	327.393	0.5	1.5	0.999
Zn	206.200	0.5	1.5	0.999
Na	589.592	0.5	1.5	0.996
B	249.677	0.5	1.5	0.999
Pb	220.353	0.1	0.3	0.999
Ni	231.604	0.3	1.0	0.999
Sn	189.927	0.5	1.5	0.989
Se	198.224	0.5	1.5	0.989
Hg	253.652	0.01	0.03	0.999
As	197.93	0.004	0.02	0.999
Cd	228.802	0.01	0.03	0.999

Table 2. The content of macroelement in tea leaves from different provinces.

Tea leave samples	Metal content (mg/kg dry weight)				
	K	Mg	Ca	Mn	Al
YB1	18074.26	2140.91	5612.49	1361.44	1087.05
YB2	15415.17	1575.68	4015.27	993.24	787.52
YB3	18595.68	2160.74	5799.63	1214.61	939.49
YB4	17630.40	2097.68	5573.42	1159.90	897.51
YB5	12494.85	1513.84	6810.78	2141.55	2416.79
YB6	12351.46	1482.18	6724.14	2149.15	2454.48
YB7	12203.49	1451.12	6593.60	2113.12	2407.45
YB8	19785.61	1433.82	3721.91	493.93	475.78
YB9	19468.94	1431.54	3706.98	477.81	456.97
YB10	19179.29	1437.98	3733.28	480.89	458.97
TQ1	18484.14	1614.04	2807.84	699.07	459.74
TQ2	19232.42	1635.56	2832.14	728.84	458.12
TQ3	20103.47	1747.94	3239.46	1127.40	739.61
TQ4	20595.16	1747.82	3194.85	1110.19	739.52
TQ5	21762.47	1754.61	3250.45	472.47	307.52
TQ6	21180.21	1721.98	3199.15	457.86	302.53
TQ7	19689.62	1443.75	3166.39	409.21	241.23
TQ8	19382.35	1418.88	3171.01	411.59	237.96
TQ9	19246.73	1439.93	3210.44	417.36	242.21

Ba, Fe, Cu, Zn, Na, B are the elements that has the content between 10 and 200 mg/kg in all the tea samples from the two provinces (Table 3). Cu plays an important role in enzyme cytochrome oxydase, ascorbic, acidaxidase, phenolase, lactase and promoting the formation of vitamin A. The Cu content in the samples from Yen Bai and Tuyen Quang varied from 12.44 to 27.94 mg/kg, which show the significant difference among the two samples set coming from the two provinces. The content of Na in tea samples from Tuyen Quang were the highest with average content was 133.83 mg/kg. The content of Na in Yen Bai tea samples were quite constant while there was large variation in Na content in Tuyen Quang tea samples.

This wide variation in metallic concentrations in the analyzed tea leaves samples could be attributed to the differences in the plant metal uptake and translocation capabilities. Metal uptake by plants depends on several factors including the plant species and their stage of grown, soil characteristic and geo-environmental conditions, climatic conditions, anthropogenic activities (pollution, industrial areas, etc.). That's why the assessment of the concentrations of elements in the different teas enabled their differentiation according to mineral composition and their classification according to geographical origin [7]. The combination of multivariate analysis techniques such as factor analysis and cluster analysis were used for data analysis can differentiate samples according to geographical origin (China, India, or Japan). In the study, the content of K, P, Mn, Fe, Cu, Co, and Cd were effective descriptors for the identification of tea samples from China, India, and Japan [7].

Table 3. The content of trace elements in tea leaves from different provinces.

Tea leave Samples	The content of trace element in tea leaves (mg/kg dry weight)								
	Na	Ba	Fe	Cu	Zn	B	Se	Sn	Ni
YB1	59.98	54.88	75.36	15.14	13.84	23.80	5.26	8.66	9.71
YB2	41.73	37.58	47.99	12.69	10.74	18.47	3.90	6.75	8.38
YB3	80.05	57.66	64.10	14.37	14.46	25.16	4.56	6.93	9.21
YB4	85.98	56.86	55.87	12.44	12.24	20.93	5.63	6.81	8.19
YB5	76.34	15.45	72.18	13.56	7.52	18.71	6.73	7.13	4.95
YB6	64.82	15.51	72.73	13.64	7.41	18.97	9.19	8.40	4.94
YB7	58.48	15.06	70.89	13.49	7.14	18.68	4.11	8.61	4.99
YB8	85.14	13.55	63.04	18.82	21.92	10.44	9.60	6.40	4.52
YB9	86.64	13.42	63.36	18.96	21.59	10.14	8.82	9.57	4.32
YB10	84.98	13.46	63.43	18.92	21.74	9.98	7.15	10.63	4.52
TQ1	228.14	12.47	107.14	22.28	24.71	12.93	10.04	7.74	6.35
TQ2	197.60	12.84	109.69	21.32	24.87	12.61	9.74	8.02	6.30
TQ3	123.17	22.48	112.48	20.09	61.54	13.85	4.10	4.27	8.29
TQ4	126.19	22.12	110.52	20.03	21.20	12.82	2.93	3.18	8.46
TQ5	87.63	9.69	76.08	19.59	26.80	15.67	5.26	6.39	4.02
TQ6	203.47	8.85	69.85	19.65	24.48	14.20	6.48	7.51	3.81
TQ7	80.81	13.43	65.71	27.65	22.65	12.24	5.88	7.31	3.02
TQ8	77.94	13.28	64.98	27.60	22.19	12.17	7.08	9.31	3.10
TQ9	79.55	13.19	65.16	27.97	22.41	12.00	8.50	7.95	3.02

The content of heavy metal in tea leaves is shown in Table 4. As was not detectable by the AAS method in this study (with the limit of detection at 0.01 ppb), Cd was found in one tea sample from Yen Bai province at 0.09 ppb and one sample from Tuyen Quang province at 0.08 ppb. Pb was found in all samples, ranging from 0.14 ppb in Yen Bai's sample to 0.67 ppb in Tuyen Quang samples. Hg was also found in all the tea leaves samples which varied from 0.02 to 0.19 ppb. Heavy metal such as Pb, Cd, and metalloid element such as As imposed adverse health effect if there is accumulation of these elements in the body through the uptake by food or drink. Compared to the study on herbal tea, this result is also similar to other results in the report 9] The content of heavy metal such as Pb, Cd, As was within the acceptable level according to the QCVN 802: 2011/BYT. There was one of the investigated sample that had the Hg content higher than the acceptable level.

Table 4. The content of heavy metal in tea leaves samples.

Tea leave Samples	The content of trace element in tea leaves (ppb)			
	As	Pb	Cd	Hg
YB1	nd	0.14	nd	0.07
YB2	nd	0.14	nd	0.07
YB3	nd	0.25	nd	0.05
YB4	nd	0.26	nd	0.05
YB5	nd	0.39	nd	0.12
YB6	nd	0.40	nd	0.11
YB7	nd	0.38	nd	0.12
YB8	nd	0.22	nd	0.13
YB9	nd	0.25	nd	0.13
YB10	nd	0.21	0.09	0.13
TQ1	nd	0.60	nd	0.19
TQ2	nd	0.60	nd	0.02
TQ3	nd	0.54	nd	0.02
TQ4	nd	0.52	nd	0.07
TQ5	nd	0.67	nd	0.08
TQ6	nd	0.65	nd	0.08
TQ7	nd	0.45	nd	0.06
TQ8	nd	0.45	nd	0.07
TQ9	nd	0.41	0.08	0.06

nd: not detected (LOD = 0.01 pp.

There is, maybe a need for a more sensitive instrument for the detection of these heavy metals [10]. To have a more comprehensive evaluation of risk, the determination of those heavy metal contents in finished made tea and tea infusion.

4. CONCLUSION

Element contents of Vietnamese green tea leaves were determined using ICP-OES, AAS non flame and GFAAS. Totally 18 elements were determined at different concentration levels, representing approximately about 10 % of total mass present in tea leave samples. Potassium presents as the highest content in all of the tea samples. This result is important for the contribution of the assessment the quality, nutrition and safety of finished tea products that is processed from these tea leaves as a raw materials.

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TÓM TẮT

XÁC ĐỊNH HÀM LƯỢNG KIM LOẠI TRONG LÁ CHÈ TRỒNG Ở YÊN BÁI VÀ TUYÊN QUANG

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Chè (được chế biến từ lá chè *Camellia sinensis* L) là loại đồ uống không cồn phổ biến trên thế giời và ở Việt Nam. Trong lá chè nguyên liệu và chè thành phẩm có những kim loại đóng vai trò quan trọng trong chất lượng của chè và những kim loại có tác dụng xấu đối với sức khỏe. Việc xác định hàm lượng các kim loại có trong chè nguyên liệu giúp có cái nhìn tổng quan về các kim loại trong chè, Tổng số 18 kim loại bao gồm Na, K, Mg, Ca, Al, Ba, Mn, Fe, Cu, Zn, B, As, Sb, Pb, Se, Cd, Ni, Sn đã được xác định trong 19 mẫu được lấy từ 2 tỉnh miền Bắc (Yên Bái, và Tuyên Quang). Các kim loại này được phân tích bằng phương pháp quang phổ phát xạ plasma cảm ứng cao tần (ICP-OES) và phương pháp quang phổ hấp thụ nguyên tử (AAS). Kết quả thực nghiệm cho thấy rằng, hàm lượng kim loại trong chè rất đa dạng trong tất cả các mẫu ở 2 tỉnh. Trong đó, K chiếm hàm lượng cao nhất trong số các nguyên tố đa lượng, tiếp theo là Mg, Ca, Mn và Al. Có sự dao động lớn giữa hàm lượng các nguyên tố vi lượng trong các mẫu chè giữa 2 tỉnh khác nhau. Hàm lượng kim loại nặng trong phần lớn các mẫu không phát hiện được, hoặc có hàm lượng thấp hơn mức cho phép theo quy chuẩn của Bộ Y tế.

Từ khóa: lá chè, *Camellia sinensis* L., kim loại, nguyên tố vi lượng, kim loại nặng.